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Lymphoid Cells in Endotoxin-Induced Production of the Amyloid-Related Serum Amyloid A Protein

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Endotoxin-treated mice exhibit a rapid rise in the level of the serum amyloid A (SAA) protein, but this effect is not observed in endotoxin-resistant C3H/HeJ mice. To evaluate the role of lymphoid cells in the production of SAA protein, C3H/HeJ mice were adoptively transfused with endotoxin-sensitive (C3H/HeN) bone marrow cells. After such adoptive transfer, endotoxin treatment of C3H/HeJ mice resulted in high serum levels of SAA protein. The ability of chimeric mice to make SAA protein correlated with the presence of endotoxin-sensitive B lymphocytes and macrophages. These findings suggest that lymphocytes and/or macrophages play an important role in initiating SAA protein synthesis after endotoxin treatment and suggest a possible mechanism by which chronic infection or inflammation leads to amyloidosis.

Secondary amyloidosis typically occurs as the result of chronic inflammatory diseases such as rheumatoid arthritis and leprosy and is characterized by the tissue deposition of the fibrilar amyloid protein AA. The serum amyloid A protein (SAA), which is thought to be a precursor of the tissue protein AA, is acutely elevated after treatment of mice with amyloidosis-inducing agents such as casein or endotoxin (lipopolysaccharide [LPS]) (9). Although the cellular origin of this protein is unknown, Linder et al. demonstrated its presence within cultured fibroblasts (8), whereas other studies reported that the liver was the first organ in which AA-like material could be detected during an acute-phase response to intraperitoneal LPS (9). These findings suggest that SAA might be a product of fibroblasts or hepatocytes. However, there is considerable evidence implicating the reticuloendothelial system in the pathogenesis of secondary amyloidosis (15), and most substances capable of elevating serum protein SAA and producing this disease are B-lymphocyte mitogens and/or macrophage activators (1, 9, 13). These findings suggest that lymphoid cells might also be involved at some stage in the production of SAA protein.

The C3H/HeJ mouse strain has been found to be resistant to all known biological effects of endotoxin (11). This unresponsiveness appears to be due to the LPS hyporeactivity of most of its cells, including B lymphocytes (11), T lymphocytes (7), macrophages (3), and fibroblasts (12). These mice exhibit a diminished elevation of serum protein SAA after endotoxin treatment (9). We have recently demonstrated the feasibility of selectively reconstituting the lymphoid cell reactivity of C3H/HeJ mice to endotoxin by the adoptive transfer of spleen cells derived from histocompatible but endotoxin-responsive (C3H/HeN) mice (4). In addition to developing endotoxin-responsive B cells and macrophages, these chimeric mice also become sensitive to the lethal effects of endotoxin. Since all of the cells and tissues except for the lymphoid cells of these chimeric mice presumably remain endotoxin unresponsive, they are a valuable model for determining the contribution of specific lymphoid cell types to the in vivo effects of endotoxin. If protein SAA were produced as a result of the direct interaction of endotoxin with fibroblasts or hepatocytes, then chimeric C3H/HeJ mice should remain unresponsive to this effect. If, on the other hand, lymphocytes or macrophages were involved, then chimeric C3H/HeJ mice should exhibit elevated protein SAA levels after treatment with endotoxin. We therefore tested the SAA protein response of chimeric C3H/HeJ mice to LPS.

Four groups of mice were tested: (i) C3H/HeJ; (ii) C3H/HeN; (iii) CeH/HeJ mice that were irradiated and reconstituted with C3H/HeJ bone marrow (B.M.) (C3H/HeJ$_x$ ← C3H/HeJ B.M.); and (iv) C3H/HeJ mice that were irradiated and reconstituted with LPS-sensitive C3H/HeN bone marrow cells (C3H/HeJ$_x$ ← C3H/HeN B.M.). Three mice in each group received either saline or *Escherichia coli* K235 (Ph) LPS intraperitoneally, and the serum levels of SAA protein were determined 18 h later.

C3H/HeJ mice did not exhibit a significant elevation of SAA protein except at the highest concentration of LPS (50 μg/mouse) (Fig. 1). The control chimeric mice (C3H/HeJ$_x$ ← C3H/HeJ B.M.) responded like the C3H/HeJ mice. In contrast, the LPS-responsive chimeric mice (C3H/HeJ$_x$ ← C3H/HeN B.M.) exhibited almost a 20-fold rise in SAA levels after receiving only 0.5 μg of LPS. This response was almost identical to that of the C3H/HeN mice.

To demonstrate a correlation between the presence of LPS-sensitive lymphoid cells and the ability to produce SAA protein in response to LPS, both the B cells and the macrophages of selected chimeric or control mice were tested for their response to LPS in vitro. The LPS-sensitive (C3H/HeJ$_x$ ← C3H/HeN B.M.) chimeric mice contained a full complement of LPS-sensitive B cells as well as macrophages, since the degree of responsiveness of their cells was identical to that of the C3H/HeN mice (Table 1). The LPS-unresponsive (C3H/HeJ$_x$ ← C3H/HeJ B.M.) control chimeric mice did not contain significant numbers of LPS-sensitive B cells or macrophages and were similar to the C3H/HeJ mice.

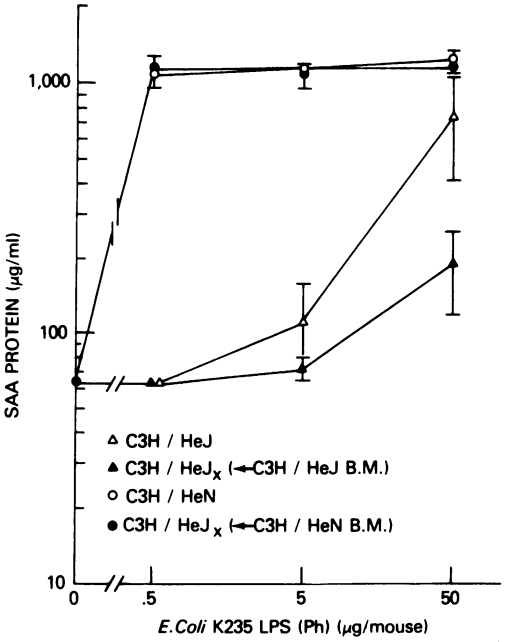

FIG. 1. *LPS-induced SAA protein production in chimeric C3H/HeJ mice. Female C3H/HeN (Division of Research Resources, National Institutes of Health) and C3H/HeJ (Jackson Laboratories, Bar Harbor, Maine) mice were maintained on autoclaved bedding in a laminar-flow unit from the age of 6 weeks and were fed autoclaved food and sterile acidified water ad libitum. For adoptive transfer, 12-week-old C3H/HeJ mice were given 700 R of X-irradiation followed within 3 h by the intravenous injection of 10 × 10^6 C3H/HeJ (C3H/HeJ$_x$ ← C3H/HeJ B.M.) or C3H/HeN (C3H/HeJ$_x$ ← C3H/HeN B.M.) bone marrow cells. Four to six weeks after adoptive transfer, mice were injected intraperitoneally with endotoxin prepared from E. coli K235 by the phenol water extraction procedure of McIntire et al. (10). Eighteen hours later mice were exsanguinated from the heart and serum was collected by centrifugation. Samples were frozen at −20°C until used. Protein SAA was measured by using a previously described solid-phase radioimmunoassay (14). Results represent the arithmetic mean ± standard error of three individual mice in each group.*

Thus, we have found that C3H/HeJ mice exhibited a nearly normal LPS-induced elevation of SAA protein after the adoptive transfer of C3H/HeN bone marrow cells. The ability to produce SAA protein correlated with the presence of LPS-sensitive B lymphocytes and macrophages in these chimeric mice. These findings strongly suggest that lymphocytes and/or macrophages play an important role in the SAA protein response.

We cannot be certain which cell type is responsible for initiating SAA protein production in response to endotoxin. Since both B cells and macrophages are LPS sensitive, and since both cell types are present in chimeric mice, it is impossible to implicate either one from available data. CBA/N mice have a selective abnormality

TABLE 1. *Analysis of B-cell and macrophage LPS responsiveness of chimeric C3H/HeJ mice*

Mice[a]	LPS responsiveness[b] of:	
	B cells (Δcpm of [^{3}H]TdR incorporated/8 × 10^5 spleen cells)	Macrophages (% killed)
C3H/HeJ	831 ± 95	4.5 ± 0.5
C3H/HeN	29,370 ± 2,720	61.5 ± 4
C3H/HeJ$_x$ (←C3H/HeJ B.M.)	1,618 ± 302	4.0 ± 0.5
C3H/HeJ$_x$ (←C3H/HeN B.M.)	34,943 ± 3,048	59.0 ± 3

[a] Selected mice from each group of chimeric or control animals were injected intraperitoneally with 3 ml of sterile thioglycolate broth, and 6 days later their spleens and peritoneal exudate cells were removed. B-lymphocyte LPS sensitivity was determined by measuring the in vitro mitogenic response of spleen cells to 5 μg of *E. coli* K235 LPS (Ph) per ml (11).

[b] Δcpm = counts per minute in LPS-stimulated cultures − counts per minute in control cultures. TdR, Thymidine. Macrophage LPS sensitivity was determined by measuring the in vitro cytotoxic effects of 50 μg of *E. coli* K235 (Ph) per ml on peritoneal macrophages (3). Results represent the arithmetic means ± standard error of three mice in each group.

in B-cell LPS unresponsiveness but have macrophages that are normally LPS sensitive (D. L. Rosenstreich, S. N. Vogel, A. Jacques, L. M. Wahl, I. Scher, and S. E. Mergenhagen, J. Immunol., in press). Therefore, the fact that these mice make normal levels of SAA protein in response to LPS (9) suggests, but does not prove, that the macrophage rather than the B cell may be responsible for this effect.

Other acute-phase serum proteins such as C-reactive protein are produced by fibroblasts or hepatocytes (6). If the SAA protein is also produced by fibroblasts or hepatocytes, as suggested by others, our data show that the synthesizing cell may not itself need to be sensitive to the effect of LPS. A population of lymphocytes or macrophages which react to LPS may in turn induce the synthesis of the protein.

We can only speculate about the mechanisms that result in production of SAA protein in response to LPS. Activated lymphoid cells release lymphokines that stimulate fibroblasts to produce collagenase and prostaglandins (2). We would hypothesize that endotoxin interacts with either B cells or macrophages in the host, causing a release of a lymphokine or monokine. This factor would then activate the synthesizing cell to elicit SAA protein production in endotoxin-treated mice. The development of casein-induced amyloidosis in normal mice can be accelerated by the adoptive transfer of lymphoid cells from mice which had been previously immunized with casein (5). In this system, it is likely that the interaction of antigen with immune lymphoid cells induces lymphokine production, which in turn causes the release of SAA protein. The findings presented in this report may therefore help explain the relationship between chronic inflammation and the development of secondary amyloidosis.

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